The Schnell Swim Test (SST) to measure motor function and recovery in spinal cord injured rats

Mirjam Gullo, Eva Hochreutener, Dina Schnell, Jeannette Scholl, Martin E. Schwab, and Lisa Schnell
Brain Research Institute, University of Zurich and Department of Biology, Swiss Federal Institute of Technology Zurich, 8057 Zurich, Switzerland, gullo@hifo.uzh.ch

Impaired functions in spinal cord lesioned rodents can be measured by a variety of different behavioral tests which range from elementary locomotor analysis to more demanding assays to analyze motor as well as sensory functions [1, 2, 3, 4, 5, 6]. Some of these tests are based on observation and rating analyses (BBB score, narrow beam), other tests require videotaping and elaborate quantifications (horizontal ladder rung walk, CatWalk®). Unfortunately, none of these tests can be strictly associated with deficits/recovery of a specific fiber system and combinations of different tests are needed to obtain reliable and unbiased motor function values.

For some tasks to be performed properly by the test animal, substantial sensory feedback such as load bearing is necessary (ladder rung etc). When using walking as a functional readout, the disadvantage is that injured animals have to perform rhythmic leg movements while having to overcome gravity. Full weight bearing has been shown to limit recovery of walking in animal models and also in patients where gait training on a treadmill with assistance in weight support is commonly used [7, 8, 9].

In this behavioral test we took advantage of the swimming motion of adult rats. Swimming is a natural behaviour of rats and the buoyancy provided by water enables them to perform locomotor movements without having to support their body weight. Since laboratory animals are normally confined to their cages, the interference with the test by self-training is not an issue. After a short training period, the animals swim without any apparent aversion and the test is hardly influenced by symptoms of stress. Motivation is provided in our setting as the animals prefer to swim straight for the platform. Swimming rats move only their hindlimbs while being supported by the buoyancy of water. Rats with different spinal cord lesions causing large or small deficits in hindlimb and tail functions, show compensatory strokes by the forelimbs. For the development of a reproducible test, velocity, forelimb strokes, hindlimb performance and tail movement were evaluated.

Velocity

The time needed to swim the marked distance of 60 cm was measured by counting the number of frames. The mean value of three runs obtained from trained animals one day before surgical intervention was taken as the baseline (32 ± frames, equal to 1.33 sec per 60 cm). This reading was assigned 100 ± %, equaling normal performance.

Forelimb strokes

The number of forelimb strokes per run was counted using video slow motion settings. Unlesioned, trained animals rarely use their forelimbs and a maximum of 2 strokes per 60 cm swimming distance can be considered the normal value. This represents a 100% performance.

Based on our results, velocity and forelimb strokes, two objectively measurable parameters, correlate already with the extent of the anatomical lesions and allow the assessment of recovery/rehabilitation after treatment. However, additional analyses can be added.

Tail Movement Score

During swimming the rat tail moves in a sinusoidal pattern with strong and regular movements. The impairment of this movement was evaluated and assigned a score from 1 to 4. A score of 4 represents normal tail movements in unlesioned animals, while a completely paretic tail with very rare twitching was given a score of 1 point, and a total absence of movement a score of 0.

Hindpaw Analysis

The normal position of the hindpaws of a swimming rat is close to the body axis. After spinal cord injury, this positioning deviates to a more lateral position and was scored accordingly. The normal swimming position of an intact rat was assigned a score of 4, while the most lateral position was given a score of 1 point. No movement of the hindlimbs (complete paresis) equals a score of 0.

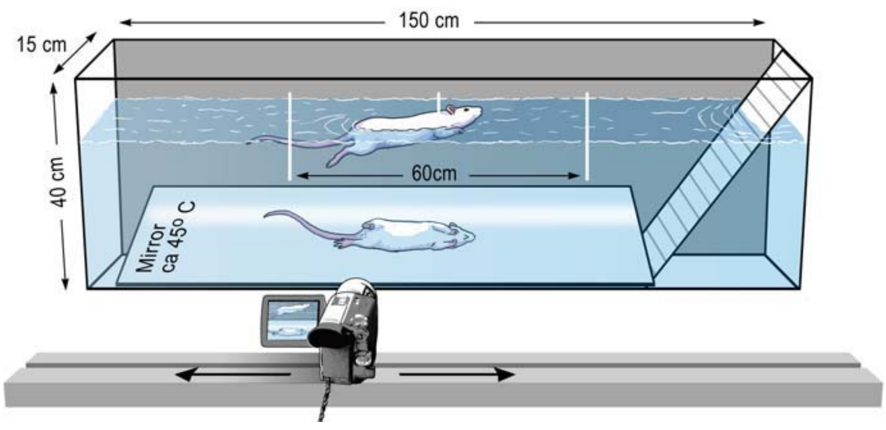

Figure 1. *Swim basin and camera*

Hindlimb-Forelimb Coordination

To judge coordination between hind- and forelimb movement, scoring criteria were used to develop a range from 5 (normal swimming pattern) to 0 (no movement of forelimbs, no movement of hindlimbs).

Inter-hindlimb coordination

Inter-hindlimb coordination was measured in frame-by-frame mode. The time stretch between the backward extension of the left and the backward extension of the right hindlimb was measured by noting the number of frames elapsed during each of 10 cycles. The differences between the numbers obtained between these 10 hindlimb strokes (5 for each leg = 9 intervals) were added and the mean of three runs converted to a percentage of the normal performance.

Summary

Swimming as a behavioral test can be used to obtain objective results in the analysis of motor function after various types of spinal cord lesions. For mild to moderate bilateral dorsal lesions, the assessment of velocity and forelimb strokes can be sufficient to obtain reliable results which correlate with the extent of the lesion or with the degree of recovery after treatment. Swimming also allowed the novel observation in rats of muscle spasms in spinal cord injured rats during the course of recovery (Gonzenbach, in prep.). Such spasms, also seen in paraplegic patients, can be measured in the in swimming rat by determining their body angle or even more accurately by electromyography (EMG). The evaluation of kinematics during swimming is currently under investigation.

References

1. Basso DM, Beattie MS, Bresnahan JC. (1995) A sensitive and reliable locomotor rating scale for open field testing in rats. *J Neurotrauma.* **12(1)**:1-21
2. Metz GA, Merkler D, Dietz V, Schwab ME, Fouad K. (2000) Efficient testing of motor function in spinal cord injured rats. *Brain Res*.**883(2),**165-77.
3. Basso DM, Fisher LC, Anderson AJ, Jakeman LB, McTigue DM, Popovich PG.(2006)Basso Mouse Scale for locomotion detects differences in recovery after spinal cord injury in five common mouse strains.*J Neurotrauma*.**23(5)**:635-59.
4. Muir GD, Webb AA. (2000) Mini-review: assessment of behavioural recovery following spinal cord injury in rats.*Eur J Neurosci.* **12(9):**3079-86
5. Hamers FP, Lankhorst AJ, van Laar TJ, Veldhuis WB, Gispen WH. (2001) Automated quantitative gait analysis during overground locomotion in the rat: its application to spinal cord contusion and transection injuries. *J Neurotrauma.* **18(2):**187-201.
6. Kunkel-Bagden E, Dai HN, Bregman BS. (1993) Methods to assess the development and recovery of locomotor function after spinal cord injury in rats. *Exp Neurol*. **119(2)**,153-64.
7. Cha J, Heng C, Reinkensmeyer DJ, Roy RR, Edgerton VR, De Leon RD. (2007) Locomotor ability in spinal rats is dependent on the amount of activity imposed on the hindlimbs during treadmill training. *J Neurotrauma.* **24(6),**1000-12.
8. Aoyagi D, Ichinose WE, Harkema SJ, Reinkensmeyer DJ, Bobrow JE. (2007) A robot and control algorithm that can synchronously assist in naturalistic motion during body-weight-supported gait training following neurologic injury. *IEEE Trans Neural Syst Rehabil Eng.* **15(3)**:387-400.
9. Dietz V, Duysens J. (2000) Significance of load receptor input during locomotion: a review. *Gait Posture.***11(2)**,102-10.